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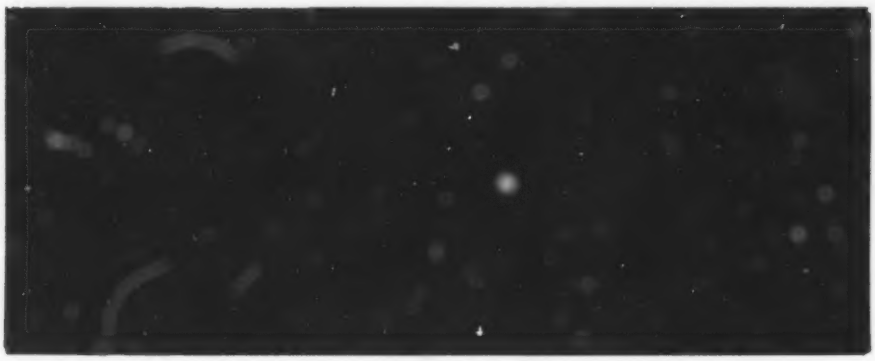

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~~Progress~~ Report: ERDA Project: 29 September 1988

"Virological Investigations into Outbreaks of Atypical Diarrhea in Calves and Piglets"

Principal Investigator: Dr. P.J.K. Durham
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Staff

A half-time technician (Mrs. Lori Hassard), was appointed under funding from the grant and has proved to be very willing and competent. Mrs. Hassard came with a background in biological sciences (Kelsey certificate in Biological Science, and previous temporary employment in the Department of Applied Microbiology, University of Saskatchewan).

Since commencing duties in April 1987, Mrs. Hassard has been trained in virological techniques related to the project including cell culture techniques, virus isolation procedures in cell culture, ultracentrifugation, electron microscopy and fluorescent antibody staining.

Another technician, Mrs. Roberta Yemen (separately funded), has also assisted with fluorescent antibody procedures and BVD virus culture.

Progress

Since commencing the project, a total of 795 feces specimens from calves, young cattle and piglets have been examined, comprising of 596 separate cases. Typical cases of BVD infection were excluded from the survey, emphasis being on purely enteric problems. Some cases held over from 1986 were also examined under the survey.

Table 1: Number of tests carried out

	Cases	Specimens	FA Tests Rota	FA Tests Corona	Isolation BVD	EM Examination
Bovine	502	666	605	559	193	220
Porcine	94	129	99	117	N/A	72

The majority of feces were from "routine" cases of diarrhea, involving small numbers of animals. In the cases, fluorescent antibody tests for rotaviruses and coronaviruses were carried out as a standard procedure. Samples were also cultured for BVD virus (by two passages in cell culture and final fluorescent antibody test) where history clinical signs or lesions were suggestive of this agent. Where outbreaks were more serious, involving substantial numbers of animals, samples were checked further by electron microscopic examination. This involved

primary clarification of fecal suspensions, ultracentrifugation through a sucrose step gradient, and electron microscopic examination of stained sediments.

Results

These are summarized in Table 2. In a number of cases, electron microscopy was used to confirm the presence of rotavirus and coronavirus where fluorescent antibody tests gave only suspicious results. On four occasions, positive fluorescent antibody tests for rotavirus in feces were not confirmed by electron microscopy (3 occasions in cattle, once in pigs).

Table 2: Viruses isolated or demonstrated.

	Rotavirus FA positive	Coronavirus FA positive	BVD virus Isolated	Enterovirus Isolated	Suspect particles (by E.M.)
Bovine	131	93	32	2	116
Porcine	2	1	N/A	0	29

*: including particles confirmed by electron microscopy.

A breakdown of the types of suspect particles detected by electron microscopy is shown in Table 3.

Table 3: Types of viruses and virus-like particles detected by electron microscopy.

		Bovine	Porcine
a):	Atypical rotaviruses (F.A. negative)	21	3
b):	Atypical coronaviruses (TGE/Bovine Coronavirus F.A. negative)	11	6
c):	Coronaviruses in adult cattle	13	N/A
d):	Bovine parvovirus	2	N/A
e):	Probable enterovirus	2	2
f):	Astrovirus	1	0
g):	Calicivirus-like (sparse numbers)	2	0
h):	Togavirus-like particles	2	0
i):	Rhabdovirus	1	0
j):	"Pin-wheel" particles	6	0
k):	Breda-like particles (numerous and typical)	42	7
l):	Breda-like particles (suspicious only)	12	11
m):	Unclassified small round viruses	2	0

DISCUSSION

Rotaviruses and Coronaviruses

The detection of numerous rotaviruses and coronaviruses was to be expected, as these viruses are commonly associated with diarrhea in young animals of most species [1,2]. The small number of cases where rotaviruses were detected in pigs partly reflects the smaller number of specimens submitted from this species, but was still considerably lower than anticipated.

Bovine Viral Diarrhea Virus

This virus was isolated from calves and young adult cattle with enteric disease.

This virus is mainly associated with infection of young adult cattle, where it may cause damage to epithelial and lymphoid tissues [3]. Most infections cause fairly mild disease, though severe disease may sometimes develop in immunotolerant cattle which carry the virus (an immunotolerant carrier state can develop in animals that are infected prenatally) [3]. Infections in calves may reflect late prenatal infection or infection soon after birth.

Atypical Rotaviruses

The rotaviruses detected under this category were sometimes present in very large numbers in the feces, so there is no doubt they were missed by the routine fluorescent antibody (FA) test. Several were rechecked by FA test, and still gave a negative result.

Rotaviruses that are antigenically distinct from "classical" rotaviruses have been previously reported in cattle and pigs from several overseas countries and in eastern Canada [5,6,7]. Because of their different antigenic makeup, they fail to react with fluorescent antibody tests for rotavirus, and hence are missed during routine virological procedures. The viruses are thought to be as pathogenic as ordinary rotaviruses.

Atypical Coronaviruses

The numbers of coronaviruses picked up by electron microscopy were never numerous, but were still thought sufficiently numerous to have been picked up by routine FA tests for TGE and bovine coronavirus. Hence it appears likely that they were of a different serotype to the routine coronaviruses.

Coronaviruses that are serologically distinct from Transmissible gastroenteritis (TGE) virus and Hemagglutinating encephalomyelitis virus (HEV) have been recorded in pigs in Europe ,Japan and in eastern Canada [8,9]. These unusual coronaviruses are known to fail to react to standard FA tests for TGE and hence are easily missed during routine investigations

into diarrheic disease in young pigs. Although it is recognized that the atypical isolates can cause substantial diarrhea, their significance in the field remains to be determined.

There do not appear to be any previous reports of coronaviruses in cattle which fail to react to the FA test for bovine coronavirus. It thus appears that these isolates may represent new serotypes of coronavirus, though further work will be required to confirm this.

Coronaviruses in Adult Cattle

One of the three outbreaks investigated here involved a fairly extensive outbreak of diarrhea in adult dairy cows held in a dairy barn. The disease spread rapidly though the animals in the barn, causing diarrhea, drop in milk production and some vague respiratory signs. Varying numbers of coronavirus were found in all of the feces samples examined by electron microscopy, no other pathogens being demonstrated, suggesting that coronavirus may have been involved in the cause of the outbreak.

Although coronaviruses are typically associated with diarrheic disease in fairly young animals, there have been a few reports of coronaviruses being associated with outbreaks of diarrhea in adult cattle [4,10,11] in New Zealand and Japan.

Breda Virus-like Particles

Breda viruses are rather difficult particles to recognize by electron microscopy. They show an appearance somewhat similar to coronaviruses, but with a finer fringe, and have some similarity to cell debris. The particles detected in the present cases appear closely similar to those described in the literature, and in many cases were present in the feces in very large numbers. In other cases, the viruses were present in small numbers only, making definite identification rather hard, and hence these particles were classed as suspicious only. Examination of one isolate showed it to have a similar density to Breda viruses described in the literature. Attempts are now underway to characterize and culture the virus, though only one report has recorded successful culture to date.

Breda viruses have been reported as causing diarrhea in calves in the USA [12] and in Europe [13], and the disease has been reproduced in experimental calves, sometimes causing severe diarrhea [12]. One report from France [13] commented that the virus was found in 60% of calves with diarrhea, suggesting that it is a common pathogen. Similar viruses have also been reported in humans with diarrhea, and in a horse.

Because the virus is rather hard to recognize by electron microscopy, and there is no FA test for the virus, it is quite possible that the virus has been missed during routine examinations, and may be more common than we realize. In several of our cases, the virus was found in association with other

viruses such as rotavirus and coronavirus, raising the possibility of mixed infections having additive or synergistic effects. It should also be noted that these particles were found in pigs with diarrhea as well as in calves.

Other Viruses and Suspect Particles

Parvoviruses, enteroviruses, caliciviruses and astroviruses were present in calf and piglet feces at low to moderate titers. Their significance remains uncertain, though these viruses have all been previously isolated from cases of diarrhea in several species of animals [14,15,16,17]. There do not appear to be any previous reports associating these organisms with enteric disease in cattle and pigs in Canada.

Parvovirus infections are well recognized thoughout the world (including Canada) as causes of severe enteric and lymphoid disease in cats and dogs. Bovine parvoviruses are generally thought to be less pathogenic, although they have been shown to be capable of causing substantial diarrhea in calves under some physiological conditions [15].

Experimental and field studies have suggested that the diseases induced by astroviruses, and caliciviruses are generally of fairly mild [12,13], though only a small number of investigations have as yet been carried out. Enteroviruses are regarded as fairly common inhabitants of the intestinal tract, and are thought to be fairly harmless in this environment, though some workers claim that some strains are capable of causing enteric disease.

The rhabdoviruses particles possessed the characteristic morphology of this virus group. They do not appear to have been previously recorded as being present in feces during enteric disease, and their presence was very unexpected. At this stage their significance remains uncertain as they were detected in only a single case.

The "pin-wheel particles" consisted of spherical particles with a diameter of about 27 nm, and with 12 very distinctive pointed projections, often with a backwards slope. Their morphology and size is very distinctive and constant, but bears no resemblance to that of known virus groups. We have only found the particles in calves, and it is no means certain that they are viruses. However at this stage there appears to be no other alternative explanation as to what they are, so it is possible they represent a new virus group. Further work will be required to clarify this.

The remaining togavirus-like particles and small round virus particles remain unclassified and are of unknown significance.

CONCLUSIONS

The most significant findings revealed in the survey were the frequent presence of rotaviruses and coronaviruses that failed to react with our standard fluorescent antibody

techniques, and the frequent presence of Breda virus-like particles.

Until we are able to develop improved fluoresent antibody techniques to detect the atypical rotaviruses and coronaviruses, it will be necessary to continue to use electron microscopy procedures to detect these viruses. However it would desirable to produce specific antisera to these agents, so as to develop improved fluoresent antibody test procedures capable of detecting the atypical organisms. These could then be incorporated into the routine tests so as to detect both typical and atypical organisms. This would be cheaper and quicker than electron microscopy for routine purposes, and would considerably improve diagnostic efficiency, with benefit to the farming community.

Breda virus-like particles were present in a considerable number of faeces, often at high titer. While the precise identity of these particles was not established, they show very close similarity to the Breda viruses described in the literature, on the basis of their size, morphology, and density. Cultural studies to date have indicated possible growth in bovine embryonic intestinal and tracheal epithelial cells. Some of the properties where these particles were detected suffered considerable losses with calf diarrhea, with no other agent being incriminated, and the organism may well play a significant role in causing the disease. In view of the considerable number of cases where these particles were seen, (often in large numbers), it is considered well worth further effort to investigate this virus further.

It is intended to continue to use electron microscopy to investigate further outbreaks of diarrhea, where standard methods fail to provide an adequate answer, and the problems are of sufficient magnitude to warrant the extra effort.. This may provide further data on the significance of the rhabdovirus, pin-wheel particles, and other viruses.

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RECOMMENDATION

The project was initiated on the basis that further funding may be considered if the results of the initial investigation appeared to justify further work. As indicated above, it would be desirable to develop improved fluorescent antibody methods to detect rotaviruses and coronaviruses, and further work is warranted on the Breda virus-like agent. We therefor request further funding to continue this work until October 1989.

PROJECT TITLE

As cited in initial research application.

KEYWORDS

Virus	Investigation	Breda virus	Rotavirus
Coronavirus	Calves	Piglets	

PERSONEL

All personnel are members of the Dept. of Vet, Microbiology, University of Saskatchewan.
1: Dr P.J.K. Durham,Principle Investigator
2: Mr R Norman, Chief technician.
3: Mrs L Hassard, Technician.
4: Mrs R Yemen, Technician.

OBJECTIVES

1: To develop improved test procedures to detect atypical rotaviruses and coronaviruses.
2: To identify the Bredavirus-like particles, to develop improved methods for their detection, and to determine their significance in causing disease in livestock.
3: To determine the frequency of occurrence of the "pin wheel" and other particles in diarrheic feces from calves and piglets, to indicate possible need for further work on these particles.

EXPERIMENTAL PROCEDURES

1: Development of test systems for atypical rotaviruses and coronaviruses.
- (a): Specific antisera to selected isolates of atypical rotaviruses and coronaviruses will be produced in laboratory animals.
- (b): Using these antisera, fluorescent antibody tests will be developed for atypical rotaviruses and coronaviruses in cattle and pigs.
- (c): The fluorescent antibody systems will then be evaluated on submitted fecal material, comparing results with fluorescent antibody procedures for standard strains of viruses, and with results of electron microscopy.

2: Determination of the significance of the Breda virus-like agent as a cause of diarrhea in calves and piglets.
- (a): Reference antisera will be obtained from researchers in the USA, enabling serological studies to be carried out on the virus. This will assist in establishing its relationship with the virus strains reported in the USA.
- (b): Antisera will be produced against the local strains of the virus, assisting procedures (a), (e) and (f).
- (c): Development of cell culture systems for the virus.
- (d): Studies of the basic physicochemical properties of the virus, to assist its viral identification.
- (e): Development of fluorescent antibody tests for the virus.
- (f): Development of an ELISA test system to detect antibodies to the virus.
- (g): Determination of the prevalence of antibody to Breda virus-like particles in calves, using the ELISA test. It

is proposed to survey up to 300 calves, some from diagnostic cases with diarrhea, and others from calves submitted for other reasons.

3: Continuation of electron microscopic surveillance of selected cases of diarrhea outbreaks in cattle and pigs, to gather more data on the prevalence of the "pin-wheel" and other agents.

LITERATURE REVIEW

See progress report above, and initial research application.

MILESTONES

The three sets of procedures will be undertaken concurrently, following the sequence outlined above. It is anticipated that we should be able to complete the objectives by October 1989.

RELEVANCE, SOCIOECONOMIC IMPACT, TECHNOLOGY TRANSFER, and INDUSTRY SUPPORT.

See initial research application.

BUDGET

1: Technician salaries for one year (Technician 1/3 time, @ $1820/ month = $7440 (Technical assistant 3 months @ $1400/ month.)	$11 640
2: Materials, supplies and incidentals.	$ 5 000
TOTAL	$16 640

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